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The effect of biochar management on soil and plant community properties in a boreal forest

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Abstract

Biochar management has been proposed as a possible tool to mitigate anthropogenic CO_2 emissions, and thus far its impacts in forested environments remain poorly understood. We conducted a large-scale, replicated field experiment using 0.05-ha plots in the boreal region in northern Sweden to evaluate how soil and vegetation properties and processes responded to biochar application and the disturbance associated with burying biochar in the soil. We employed a randomized block design, where biochar and soil mixing treatments were established in factorial combination (i.e., control, soil mixing only, biochar only, and biochar and soil mixing; $n = 6$ plots of each). After two growing seasons, we found that biochar application enhanced net soil N mineralization rates and soil NH_4^+ concentrations regardless of the soil mixing treatment, but had no impact on the availability of NO_3^- , the majority of soil microbial community parameters, or soil respiration. Meanwhile, soil mixing enhanced soil NO_3^- concentrations, but had negative impacts on net N mineralization rates and several soil microbial community variables. Many of the effects of soil mixing on soil nutrient and microbial community properties were less extreme when biochar was also added. Biochar addition had almost no effects on vegetation properties (except for a small reduction in species richness of the ground layer vegetation), while soil mixing caused significant reductions in graminoid and total ground layer vegetation cover, and enhanced seedling survival rates of *P. sylvestris*, and seed germination rates for four tree species. Our results suggest that biochar application can serve as an effective tool to store soil C in boreal forests while enhancing NH_4^+ availability. They also suggest that biochar may serve as a useful complement to site preparation techniques that are frequently used in the boreal region, by enhancing soil fertility and reducing nutrient losses when soils are scarified during site preparation.

Keywords: biochar management, boreal forest, carbon sequestration, charcoal, forest regeneration, ground layer vegetation, humus, nitrogen mineralization, nutrients, soil carbon efflux

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Introduction

Since the start of the industrial revolution, atmospheric CO_2 concentration have risen from 280 to above 400 ppm, with some models predicting concentrations will reach 1000 ppm by the end of the century (IPCC, 2013). These emissions have already led to increases in mean global temperatures and caused associated changes in precipitation in many regions (IPCC, 2013). Due to the current and potential impacts of these changes on society, there is substantial interest in developing sustainable land management systems aimed at sequestering atmospheric carbon (C) while simultaneously maintaining and optimizing other ecosystem services such as commodity production (Burney *et al.*, 2010; Palm *et al.*, 2010; Post *et al.*, 2012). Biochar management has been proposed as an approach that may potentially accomplish these goals simultaneously, and as such has gained widespread attention among researchers and policy-makers throughout the world (Lehmann & Joseph, 2009). Biochar management involves intentionally charring biomass and burying it, with the purpose of directly storing C in the soil, while at the same time enhancing soil quality and plant productivity (Lehmann, 2007a,b; Sohi, 2012). It has been proposed that a significant proportion of anthropogenic CO_2 emission could be mitigated if biochar management were adopted globally (Lehmann, 2007b); however, key data are lacking regarding its impacts on soils and plants in a variety of ecosystem types (Sohi, 2012; Jeffery *et al.*, 2013; Cusack *et al.*, 2014).

The vast majority of biochar research has to date been conducted in tropical and temperate agro-ecosystems (Atkinson *et al.*, 2010; Jeffery *et al.*, 2011; Lehmann *et al.*, 2011; Clough *et al.*, 2013). Studies from these systems

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have shown that a wide variety of charring techniques can be used to stabilize a range of organic residues, by altering their C chemistry (e.g., structural components such as cellulose or lignin) into irregular or graphitic molecular structures (Steiner *et al.*, 2007; Nelissen *et al.*, 2012; Clough *et al.*, 2013). Many microbes lack efficient enzyme systems to mineralize charred materials, allowing some charred materials to remain stable over centennial to millennial time scales (Glaser *et al.*, 2001; Preston & Schmidt, 2006). While it's very high C content (usually >70% C) and recalcitrance have the potential to contribute to long-term soil C storage, numerous studies have also shown that biochar can enhance soil fertility and plant growth. For instance, biochar often has high concentrations of available nutrients (e.g., NH_4^+ , PO_3^- , Ca^{+2}, and Mg^{+2}) on its surfaces, which can have fertilization effects over short time-scales (Gundale & Deluca, 2006; Chan & Zhihong, 2009; Jeffery *et al.*, 2011; Pluchon *et al.*, 2014). Biochar has also been shown to enhance nutrient availability over longer time scales by enhancing nitrogen (N) mineralization or nitrification (DeLuca *et al.*, 2006; Ameloot *et al.*, 2015) as a result of enhanced microbial growth and activity (Zackrisson *et al.*, 1996; Lehmann *et al.*, 2011) and by reducing soil nutrient losses due to its high ion exchange capacity (Atkinson *et al.*, 2010; Brewer *et al.*, 2011). Numerous recent studies have shown that the positive effects of biochar on soil fertility can result in enhanced plant growth (Wardle *et al.*, 1998; Lehmann, 2007a; Jeffery *et al.*, 2011; Biederman & Harpole, 2013; Liu *et al.*, 2013), thereby having an indirect positive effect on net ecosystem C uptake.

A very limited amount of research on biochar has been conducted in forested ecosystems, including boreal forests that cover approximately 11% of the terrestrial land surface area and serve as one of the largest stores of terrestrial C (Sabine *et al.*, 2004). A wide range of intensive forest management approaches are currently being investigated in forest ecosystems to either sequester C or produce biofuel to offset fossil fuel demand (e.g., stump harvesting, establishment of short rotation age exotic tree plantations, and biochar management) (Walmsley & Godbold, 2010; Lindholm *et al.*, 2011; Sabatti *et al.*, 2014). One aspect of biochar management that remains poorly understood in forested systems is whether biochar can enhance soil fertility without stimulating loss of existing soil C. Several aspects of biochar management have the potential to cause unintended C losses, including physical disruption of soil during burying (Jones *et al.*, 2010) and positive effects of biochar on soil microbial growth and activity (Pietikainen *et al.*, 2000; O'Neill *et al.*, 2009; Liang *et al.*, 2010). Previous studies have suggested that the addition of charcoal to boreal soils can accelerate the decomposition of organic matter (Wardle *et al.*, 2008); however, studies have yet to discern the effects of biochar itself vs. those of physical disturbance on soil C losses. An additional aspect of biochar management that remains poorly understood in forested ecosystems is its potential impact on ground layer vegetation composition, as well as the regeneration of forest tree species (Pluchon *et al.*, 2014). In contrast to most agricultural systems, a wide variety of plant species often co-occur in forested environments and these can exhibit considerable variation in their functional traits (Hobbie, 1996; Cornwell *et al.*, 2008) and relationships with local-scale soil fertility (Wardle *et al.*, 2012). Thus, fertility changes associated with biochar application may impact ground layer species composition, which could in turn influence tree seedling establishment and growth rates. Such effects are relevant to understanding ecosystem C sequestration because as forests develop trees eventually serve as the largest aboveground C pool. To our knowledge, the impact of biochar management on soil nutrient and C dynamics, as well as ground layer vegetation composition and tree seedling establishment, has not been evaluated at an operational scale in a forested environment (Robertson *et al.*, 2012; Sohi, 2012; Cusack *et al.*, 2014).

We conducted a large-scale experiment in the boreal forest zone of northern Sweden to understand how biochar management impacts on a wide range of soil and plant properties. We applied biochar and soil mixing to field plots in a factorial combination (i.e., control, biochar only, mixing only, and biochar and mixing combined) so that we could discern the impact of biochar addition by itself (i.e., applied on the soil surface) vs. the disturbance impact caused from incorporating it into the soil. We hypothesized that: 1) the addition of biochar will enhance nutrient availability and mineralization rates, microbial biomass, and soil respiration rates independently of the soil mixing treatment; 2) soil mixing alone also will enhance nutrient availability and mineralization rates, and soil respiration, but decrease total microbial biomass and fungal to bacteria ratios, and further, that soil mixing will enhance any effects of biochar on these properties through bringing the biochar in closer contact with the soil; 3) enhanced short-term nutrient availability caused by the individual and synergistic effects of biochar, and mixing treatments will result in significant changes in ground layer plant species composition, with higher nutrient availability leading to greater plant cover and dominance of graminoid species, which have high relative growth rates; and 4) greater ground layer vegetation cover associated with biochar and mixing treatments will correspond with reduced germination of tree seeds and survival rates of tree seedlings, due to competitive suppression. Testing these hypotheses in combination will provide

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valuable insights into the impacts of biochar management on ecosystem functioning in forest ecosystems.

Materials and methods

Study site and experimental design

The experiment was performed at the Åheden research area within the Svartberget Experimental Forest (64°14′N, 19°46′E, 175 m above sea level) in northern Sweden. Soil at the site is a fine sandy Typic Haplocryod (FAO, Cambic Podzol) formed from silty glacial outwash sediment. The annual mean air temperature at the site is + 1.0 °C. Snow usually covers the ground from the end of October to late April. Mean annual precipitation is approximately 600 mm, of which half falls as rain and half as snow (Gundale *et al*., 2011a). Prior to the start of the experiment, the experimental site was covered with a closed tree canopy consisting of ~60-year-old Scots pine (*Pinus sylvestris*). Prior to the experiment, the ground layer vegetation consisted primarily of ericaceous shrubs, mainly *Vaccinium vitis-idaea* and *Calluna vulgaris*, and of mosses and lichens (predominantly *Pleurozium schreberi*, *Dicranum* sp., *Cladina rangiferina*, and *Cladina arbuscula* (Gundale *et al*., 2011a).

In October, 2010, a 5-ha area within the experimental forest was clear felled using standard forestry practice, and tree boles and branches were removed from the site. The following summer (i.e., July 2011), soil C stocks were estimated at the site by measuring the dry mass and C concentration on 24 homogenized soil cores, which were 20 cm in depth and included the organic horizon and mineral soil. These measurements revealed that the average bulk density across these soil depths was 1.00 g cm^{-3}, and in total contained 38.8 Mg of C ha^{-1} (standard error = 2.82) prior to the establishment of treatments. In October, 2011, we established 24 0.05-ha (22.4 × 22.4 m) experimental plots, with a 5-meter buffer zone between each plot. The plots were arranged into six blocks, with each of four treatments (control, biochar addition only, soil mixing only, and biochar addition followed by soil mixing) randomly assigned to one of four plots within each block. Because stumps were an obstacle for effectively implementing the soil mixing treatment, we used an excavator which we positioned outside of each plot to vertically lift and remove all stumps from every plot. This removal caused a local disturbance at the site of each stump, but left the organic horizon intact in most of the plot. We note that stump harvesting is an active area of research in the Nordic region because of its considerable potential as a source of biofuel (Walmsley & Godbold, 2010; Lindholm *et al*., 2011). Following stump removal, the excavator was fitted with a 1000-L excavating bucket (Fig. 1), which was used to spread charcoal and establish the mixing treatments.

For the charcoal treatments, each plot received 500 ± 15 kg of biochar, equivalent to 10 tons biochar ha^{-1} dry weight, which is within the range of addition rates commonly used in biochar experiments in agro-ecosystems (Liu *et al*., 2013). This is approximately three times greater than the highest quantities of black C that have been estimated in pine forests in northern Sweden (Ohlson *et al*., 2009). The biochar was produced by a local company (Vindelkol AB, Vindeln Sweden) and is sold

Fig. 1 The excavator used to apply biochar and mix soils in an experiment consisting of four treatments; a control, biochar only, soil mixing only, and biochar plus soil mixing (n = 6 of all treatment combinations).

and marketed as 'Terra Preta,' a product intended as a soil amendment (www.vindelkol.se). The biochar was made primarily from the wood and bark of *P. sylvestris*, and a small portion of *Picea abies* and *Betula pendula*. Analysis of the biochar showed it had a pH of 8.04 and contained concentrations of extractable PO_4^- -P, NO_3^-, and NH_4^+ of 1.26, 0.14, and 1.38 mg kg^{-1}, respectively, and a C concentration of 74%, and a P concentration of approximately 300 mg kg^{-1}. Once biochar was applied to each plot assigned for biochar addition, the excavator was used to mix the soil in all plots assigned for soil mixing. This was done with the excavator by removing the soil to a depth of approximately 30 cm, piling it within the plot, and then evenly spreading and smoothing throughout the plot using the steel teeth on the front edge of the excavator bucket. The same mixing and spreading procedure was used in the mixing only treatment, whereas control plots only received disturbance associated with tree and stump removal. In the spring of 2012, a fence was built around the entire experimental area to exclude large herbivores (e.g., moose), and the site was planted with approximately 10-cm-tall containerized *P. sylvestris* seedlings provided by a commercial nursery, with plants arranged in a 2 × 2 m grid pattern.

Soil chemistry measurements

We assessed the relative availability and mineralization of NH_4^+ , NO_3^- and PO_4^--P ions by extracting and analyzing bulk soil samples and by conducting an *in situ* mineralization assay. In June, 2013, five bulk soil samples were collected from within each plot from each of two depths (0–10 and 10–20 cm). These samples were collected using a 49-mm-diameter soil corer, from the plot center and from the four midway positions between each plot corner and the plot center. Samples were placed in coolers, returned to the laboratory, and sieved (2 mm). The five subsamples from each depth within each plot were then bulked, and a 20-g homogenized subsample was extracted with 50 mL of 1 M KCl. At the same time these soil samples were collected, an additional identical set of 5 cores

were collected directly adjacent to the first set, placed in polyethylene bags, and returned to the same position from where they originated. These samples were left in the soil for 2.5 months, after which they were collected, bulked, processed, and extracted in the same way as the original set. Concentrations of NH_4^+ , NO_3^- , and PO_4^- -P were measured on extracts of each soil type (i.e., bulk soil and incubated soils) using standard colorimetric techniques on an autoanalyzer III (Omni Process, Solna, SE) (Gundale *et al*., 2008, 2011a; Wardle *et al*., 2013). Approximately 5 g of each soil sample was separated for the determination of gravimetric water content, which allowed nutrient concentration measures on all soil extracts to be reported on a soil dry weight (d.w.) basis. For each plot, net N mineralization and P mineralization (mg g^{-1} soil dry weight per day) were calculated as the difference in inorganic N (NH_4^+ plus NO_3^-) or P (PO_4^- -P) concentrations between the nonincubated and incubated samples, and net nitrification was calculated as the difference in NO_3^- concentrations, and divided by the incubation period (Eno, 1960; Gundale *et al*., 2011a).

In May, 2013, we placed five mixed bed ionic resin capsules (PST1 capsule, Unibest, Bozeman, USA) in each plot adjacent to the same five sampling locations described above (Gundale *et al*., 2008). The capsules were inserted at a 45° angle, with a final depth of 5 cm beneath the surface of the humus, and always at least 20 cm from where the soil was sampled. Ionic capsules were collected in October 2013 and extracted using three consecutive rinsing of 10 mL 1 M KCl (30 mL total) (Gundale *et al*., 2011b, 2014). Extracts from these capsules were analyzed as described above for NH_4^+ , NO_3^-, and PO_4^- -P, and nutrient concentrations were reported on a per capsule basis.

Soil pH was measured on each sample by creating a slurry solution consisting of a 1 : 1 mixture of dry soil and deionized water. After combining the soil and water, the slurry was allowed to equilibrate for 4 h prior to measuring pH.

Soil microbial community measurements

We estimated the relative active soil microbial biomass using the substrate-induced respiration (SIR) method (Anderson & Domsch, 1978), as modified by Wardle (1993) and Gundale *et al.* (2011b). Measurements were made using a composite of 20 g (d.w.) soil sample from each plot at both depths (i.e., 0–10 and 10–20 cm). Each sample was placed into a 100-mL glass bottle, adjusted to a water content of 125% (d.w. basis), injected with a glucose solution, and then sealed. Evolution of CO_2 between 1 and 3 h following glucose addition was then determined by injecting 5 mL subsamples of headspace gas into an infrared gas analyzer. The difference in respiration between the two sample times is an effective proxy of soil microbial biomass (Anderson & Domsch, 1978).

In addition to SIR, we used phospholipid fatty acid (PLFA) analysis to measure soil microbial community structure (Bligh & Dyer, 1959). The PLFAs were extracted from 1 g of freeze-dried soil samples using a modification of the Bligh and Dyer method (Bligh & Dyer, 1959; McIntosh *et al*., 2012; Wardle *et al*., 2013). Extracts containing PLFAs were analyzed on a Perkin Elmer Claris 500 Gas Chromatograph (Massachusetts, USA), as described elsewhere (McIntosh *et al*., 2012; Wardle *et al*., 2013). The abundance of identified PLFAs was reported as micromoles per gram of organic matter using conventional nomenclature and subsequently converted to relative abundance. Different types of PLFAs represent different components of the soil microflora. The PLFAs 18:1ω9 and 18:2ω6 were used to estimate the contribution of fungi, while the branched fatty acids 10me16:0, 10me17:0 and 10me18:0 were used to estimate actinobacteria. Bacterial PLFAs including i-15:0, α-15:0, 15:0, i-16:0, 16:1ω9, 16:1ω7t, i-17:0, cy-17:0, α-17:0, 18:1ω7, cy-19:0 were used to represent total bacteria. Gram-positive bacteria were represented by branched fatty acids i-15:0, α-15:0, i-16:0, i-17:0 and α-17:0, while cy-17:0, cy-19:0 and 18:1ω7 were used to represent gram-negative bacteria.

Soil respiration measurements

We measured total soil respiration (i.e., autotrophic and heterotrophic respiration combined) on six separate measurement events between May 29 and September 13, 2013, with approximately 3 weeks between each event. Each measurement event required 2 days, with measurements occurring between 9:00 and 16:30 hours. The order in which plots were measured was changed between sampling events with the intention of dispersing any diurnal variation in measurements equally among blocks and treatments. The measurements were made by installing five cylindrical collars (each 25 cm diameter, 10 cm high) in each 0.05-ha plot in early May 2013. The collars were inserted 1 cm into the soil surface, and all aboveground vegetation inside and 5 cm around each collar was manually removed. The collars were allowed to equilibrate for 17 days after they were set up before the first measurement. The height from soil surface to the rim was measured in four cardinal directions within each collar to calculate the headspace volume. Soil respiration (CO_2 efflux) was then measured by sealing the headspace within an opaque plexiglass lid fitted with a portable infrared gas analyzer (CARBOCAP model GMP 343, Vaisala, Finland). From each collar, CO_2 concentrations were recorded every 15 s for 3 min. During each measurement, we recorded the headspace air temperature using a digital thermometer probe. Soil respiration within the headspace of each chamber was calculated using a linear regression of CO_2 concentration vs. time, with the slope of the regression indicating the CO_2 efflux. Estimated values were subsequently adjusted for variation in headspace volume and air temperature, and converted to a soil surface area basis, resulting in units of μmol CO_2 m^{-2} s^{-1}, as described by Hasselquist *et al*. (2012).

Vegetation measurements

Total vegetation and graminoid cover in each plot were estimated in August, 2013, by randomly placing 20 0.5 × 0.5 m quadrats within the plot and visually estimating percent cover values within each quadrat. To assess plant community composition within each plot, we used point quadrat analysis in August, 2013, as described by Wardle *et al.* (2003) for each of three randomly positioned 0.5 × 0.5 m quadrats. The percent cover of all plant species (including vascular plants and

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mosses) within each quadrat was estimated by downwardly projecting 100 points, and recording all vegetation intercepts for each species by each projection (Wardle *et al*., 2003). Further, survival of planted *P. sylvestris* seedlings in each plot was measured by visually determining the proportion of these seedlings that were still alive in September 2013.

To explore the effects of treatments on tree seed germination, we sowed seeds of the native Swedish tree species *Betula pubescens*, *P. sylvestris*, and *P. abies*, and the introduced Canadian tree species, *Pinus contorta*. In each plot, we set up five subplots directly adjacent to each location where soil measurements were made. Each subplot consisted of four parallel 20-cm-long lines, with 10 cm separating each line. Each line was randomly assigned to one of the four tree species and planted with 50 seeds each (i.e., ~0.5 cm between each seed). Seeds were sowed on June 12 and 13, 2013, and germinated seeds were counted on June 28, July 9, July 31 and September 13, 2013 (i.e., 15, 26, 48 and 92 days after sowing, respectively). A wire mesh (1 × 1 cm mesh size) was placed above each subplot for the duration of the experiment to protect seeds and seedlings from consumption by birds and small mammals.

Statistical analysis

For all variables, we considered the plot to be the unit of replication, meaning that multiple measurements made within each plot at the same time were always averaged in order to generate plot level values. When variables were measured at multiple times (i.e., respiration and seedling germination), or at multiple soil depths, plot level averages were made separately for each measurement time or depth. All data were subsequently analyzed using analysis of variance (ANOVA). For variables measured at both sampling depths (i.e., SIR, PLFA, pH, extractable soil nutrients, and soil nutrient transformations variables), data were analyzed using a three-factor split plot ANOVA, with soil depth (0–10 or 10–20 cm) serving as a split-plot factor within biochar (added or not added) and mixing (mixed or not mixed) as main factors. Soil respiration data were analyzed using repeated-measures ANOVA, with biochar and mixing serving as fixed factors, and sampling time serving as the repeated measure. The final seedling germination rate measured 92 days after sowing was analyzed using a three-factor ANOVA, with seedling species serving as a subplot factor within biochar and mixing treatments. The remaining data (i.e., resin capsule and all other vegetation data) were analyzed using two-factor ANOVA, with biochar and mixing serving as fixed factors. Prior to two-way ANOVA, vegetation community composition data were subjected to principal components analysis (PCA), and scores from the first and second principal component axes were analyzed using two-way ANOVA. For all ANOVAs described above, block was initially included as a random factor within each ANOVA model and was removed whenever significant block effects were not present (Zar, 1999). Whenever ANOVAs revealed significant interactive effects among factors, data were subsequently analyzed using *post hoc* Student–Newman–Keuls tests to identify pairwise differences among treatment combinations, or Student's t-tests to compare two treatments within a given soil depth. All data were

Table 1 The *F*- and *P*-values derived from three-factor split plot ANOVAs comparing the main and interactive effects of biochar (added or not added), soil mixing (mixed or not mixed), and soil depth (0–10 or 10–20 cm) on extractable pools of NH_4^+ -N, NO_3^- -N, PO_4^- -P, pH, net nitrogen mineralization (Nmin), net nitrification (Nnit), net phosphorous mineralization (Pmin), and substrate induced respiration. Bolded *F*- and *P*-values indicate a significant effect at $P = 0.05$

	Mixing (M)*		Biochar (B)*		Depth (D)*		B × M*		B × D*		M × D*		B × M × D	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Extractable NH_4^+ (mg kg^{-1} soil d.w.)	0.8	0.376	**10.5**	**0.003**	3.4	0.075	0.1	0.712	1.0	0.334	1.4	0.250	0.0	0.986
Resin NH_4^+ (mg $capsule^{-1}$)	0.9	0.368	**4.3**	**0.050**	–	–	0.2	0.702	–	–	–	–	–	–
Extractable NO_3^- (mg kg^{-1} soil d.w.)	**7.5**	**0.010**	0.1	0.716	**10.4**	**0.004**	1.0	0.316	0.9	0.358	2.1	0.160	0.7	0.419
Resin NO_3^- (mg $capsule^{-1}$)	1.3	0.268	0.3	0.594	–	–	**5.7**	**0.031**	–	–	–	–	–	–
Extractable PO_4^- (mg kg^{-1} soil d.w.)	2.6	0.118	0.0	0.953	**6.8**	**0.013**	1.0	0.322	0.0	0.985	2.7	0.111	0.9	0.343
Resin PO_4^- (mg $capsule^{-1}$)	**34.3**	**0.000**	4.2	0.058	–	–	**11.1**	**0.005**	–	–	–	–	–	–
Nmin (mg kg^{-1} soil d.w. day^{-1})	**13.6**	**0.001**	**4.5**	**0.041**	**29.8**	**0.000**	**5.5**	**0.025**	2.2	0.146	**5.1**	**0.030**	1.7	0.202
Nnit (mg kg^{-1} soil d.w. day^{-1})	1.4	0.245	0.6	0.453	0.6	0.441	0.6	0.458	0.8	0.379	0.3	0.575	0.3	0.575
Pmin (mg kg^{-1} soil d.w. day^{-1})	1.7	0.196	2.3	0.140	0.1	0.787	0.7	0.427	0.1	0.744	0.0	0.866	0.1	0.815
pH	0.9	0.342	2.0	0.164	**37.2**	**0.000**	0.3	0.565	0.3	0.565	**6.0**	**0.020**	0.7	0.423

*Degrees of freedom: 1,5.

Table 2 The *F*- and *P*-values derived from three-factor ANOVAs comparing the main and interactive effects of biochar (added or not added), soil mixing (mixed or not mixed), soil depth (0–10 or 10–20 cm) on soil microbial variables, including substrate induced respiration (SIR), and phospholipid fatty acid (PLFA) data, including total, fungal, and bacterial PLFAs, and the fungal to bacterial ratio. Bolded *F*- and *P*-values indicate a significant effect at $P = 0.05$

	Mixing (M)*		Biochar (B)*		Depth (D)*		B × M*		B × D*		M × D*		B × M × D*	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
SIR (μg C g^{-1} h^{-1})	**15.2**	**0.000**	2.5	0.121	**129.5**	**0.000**	**21.6**	**0.000**	0.6	0.432	**17.4**	**0.000**	**11.5**	**0.002**
Total PLFA (nmol g^{-1} O.M.)†	**12.9**	**0.001**	1.7	0.204	**83.5**	**0.000**	**4.3**	**0.046**	0.2	0.627	**16.9**	**0.000**	2.0	0.170
Fungi (nmol g^{-1} O.M.)	**14.1**	**0.001**	2.8	0.102	**87.1**	**0.000**	3.3	0.077	0.7	0.399	**19.0**	**0.000**	1.6	0.210
Bacteria (nmol g^{-1} O.M.)	**12.0**	**0.001**	1.5	0.226	**80.2**	**0.000**	**4.6**	**0.039**	0.3	0.623	**15.7**	**0.000**	2.3	0.142
Gram + (nmol g^{-1} O.M.)	**11.6**	**0.002**	1.9	0.183	**85.0**	**0.000**	**4.4**	**0.044**	0.3	0.571	**15.8**	**0.000**	1.9	0.174
Gram - (nmol g^{-1} O.M.)	**11.9**	**0.001**	1.3	0.271	**73.5**	**0.000**	**4.7**	**0.038**	0.2	0.686	**15.0**	**0.000**	2.4	0.134
Actinobacteria (nmol g^{-1} O.M.)	**12.9**	**0.001**	2.0	0.170	**84.0**	**0.000**	**4.7**	**0.038**	0.2	0.682	**16.3**	**0.000**	2.0	0.163
Fungal to bacterial ratio	0.5	0.933	**5.2**	**0.029**	**38.1**	**0.000**	1.3	0.266	0.1	0.933	**6.8**	**0.013**	0.1	0.813

*Degrees of freedom: 1,5.
†Organic Matter (O.M.).

Table 3 The degrees of freedom, *F*- and *P*-values from a repeated-measures ANOVA evaluating the main and interactive effects of biochar (added or not added), soil mixing (mixed or not mixed), sampling time, and their interactions on soil respiration, expressed as μmol m^{-2} s^{-1}. Bolded *F*- and *P*- values indicate a significant effect at $P = 0.05$

	df	*F*-values	*P*-values
Mixing	1,5	**7.9**	**0.006**
Biochar	1,5	2.8	0.096
Time	3,15	**12.1**	**0.000**
Mixing*Biochar	1,5	2.5	0.117
Mixing*Time	3,15	**2.8**	**0.019**
Biochar*Time	1,5	0.1	0.992
Biochar*Mixing*Time	3,15	0.8	0.578

analyzed using SPSS version 21 (IBM, New York, NY, USA) except for the PCA for which Primer version 6 software (Luton, UK) was used. Data were first evaluated for assumptions of normality and homoscedasticity prior to ANOVA and were always found to meet these assumptions.

Results

Soil response variables

Numerous soil variables were responsive to biochar addition, soil mixing, and soil depth, as well as their interactions (Tables 1, 2, and 3). For soil depth, extractable PO_4^- concentrations (Fig. 2a), net N mineralization (Fig. 2b), and all soil microbial community parameters (Fig. 3) decreased with depth, whereas extractable NO_3^- concentrations increased with depth (Fig. 2c).

Biochar addition caused a significant increase in net N mineralization (Fig. 2b, Table 1), extractable NH_4^+ concentrations (Fig. 2d, Table 1), resin-sorbed NH_4^+ concentrations (Fig. 4a, Table 1), and the fungal:bacterial ratio (Fig. 3e, Table 2). Biochar had no significant main effects on any other response variable nor were there any significant interactions between biochar and soil depth.

Soil mixing had a significant main effect on many response variables. Mixing caused a significant decrease in net N mineralization rates (Fig. 2b, Table 1), SIR, total PLFAs, the abundance of all microbial functional groups (Fig. 3a–d, Table 2), soil respiration rates (Supplementary Fig. 1, Table 3), and an increase in soil NO_3^- concentrations (Fig. 2c, Table 1). For several variables, soil mixing also showed an interactive effect with soil depth (Table 1, 2). For net N mineralization, this interaction occurred because the negative effect that soil mixing had on N mineralization was more pronounced in the surface compared to deeper soil (Fig. 2b). Likewise, for all microbial response variables (Table 2), the negative impact of mixing was more pronounced in surface relative to deeper soil (Fig. 3a–e; data for Gram+, Gram-, and actinobacteria not graphically depicted). For soil pH, the interactive effect occurred because mixing increased soil pH in the surface ($pH = 4.88 \pm 0.054$ and 5.17 ± 0.036 in the unmixed and mixed soil, respectively), but not in the deeper soil layer (5.41 ± 0.036 regardless of treatment). For soil respiration, the effect of mixing interacted significantly with time (Table 3), with negative effects more pronounced in the middle compared to the beginning or end of the growing season (Fig. S1).

Numerous soil variables responded to the interactive effect of biochar and mixing, and one additional variable (SIR) responded to a three-way interactive effects

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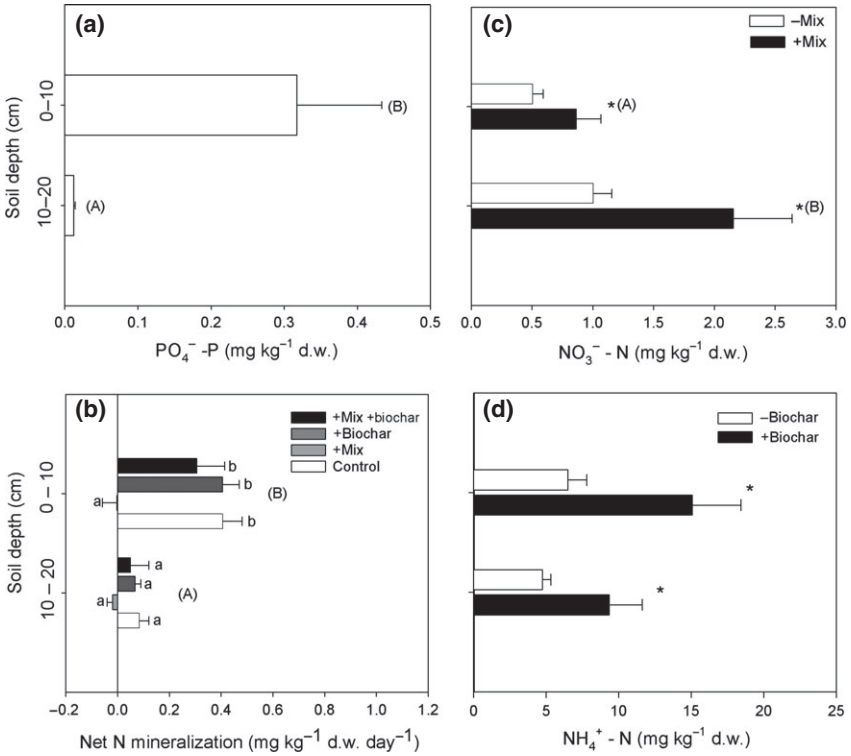

Fig. 2 The mean (±SE) extractable soil PO_4^{3-} concentrations in response to soil depth (a), net N mineralization rates in response to soil depth and biochar and soil mixing treatments (b), NO_3^- concentrations in response to soil depth and soil mixing (c), and soil NH_4^+ in response to soil depth and biochar application (d). Within each panel, different capital letters (A or B) above bars or bar groups indicate a significant difference between the soil depths; lower case letters (a or b) indicate significant differences across all bars as indicated by *post hoc* Student–Neuman–Keuls tests, and stars (*) indicate significant differences between the two bars within each soil depth, as determined using *post hoc* Student t-tests.

of biochar, mixing, and soil depth (Table 1 and 2). For net N mineralization (Fig. 2b), all microbial parameters except fungi and the fungal to bacteria ratio (Fig. 3a–e), and resin-sorbed NO_3^- and $H_2PO_4^-$ (Fig. 4b,c), two-way interactive effects between biochar and mixing occurred because the impacts of soil mixing on these variables were less severe when biochar was also added. Likewise, the three-way interactive effect found for SIR was due to the negative effects of soil mixing being less severe when biochar was also added, but only in the surface soil (Fig. 3a).

Vegetation response variables

The addition of biochar had a significant main effect on only one vegetation variable, that is, plant species richness, which declined when biochar was added (Fig. 5d, Table 4). Soil mixing had a negative impact on total plant cover and graminoid cover (Fig. 5a, Table 4). Mixing also reduced PCA axis 2 scores (Fig. 5b, Table 4), where lower scores represented greater abundance of *Vaccinium vitis-idaea* and *Calluna vulgaris*, and higher scores represented greater abundance of *Deschampsia flexuosa* and *Epilobium angustifolium* (loading scores −0.44, −0.22, 0.34, and 0.76, respectively). In contrast, pine seedling survival was significantly enhanced by soil mixing (Fig. 5c, Table 4), as was the germination percentage of all four tree species (Fig. 6, Table 5). For tree seed germination, the positive effect of mixing on germination differed among the four species (i.e., indicated by a significant mixing by species interaction; Table 5). These interactive effects occurred because the positive effect of mixing was less pronounced for *B. pendula* compared with the three conifer species (Fig. 6). One variable, that is total vegetation cover, was also affected by the interaction of biochar and mixing, whereby the negative effect that mixing had on total cover was more severe when biochar was added compared to when it was not added (Fig. 5a).

Discussion

Consistent with our first hypothesis (i.e., that addition of biochar would enhance nutrient availability and mineralization rates, microbial biomass, and soil respiration

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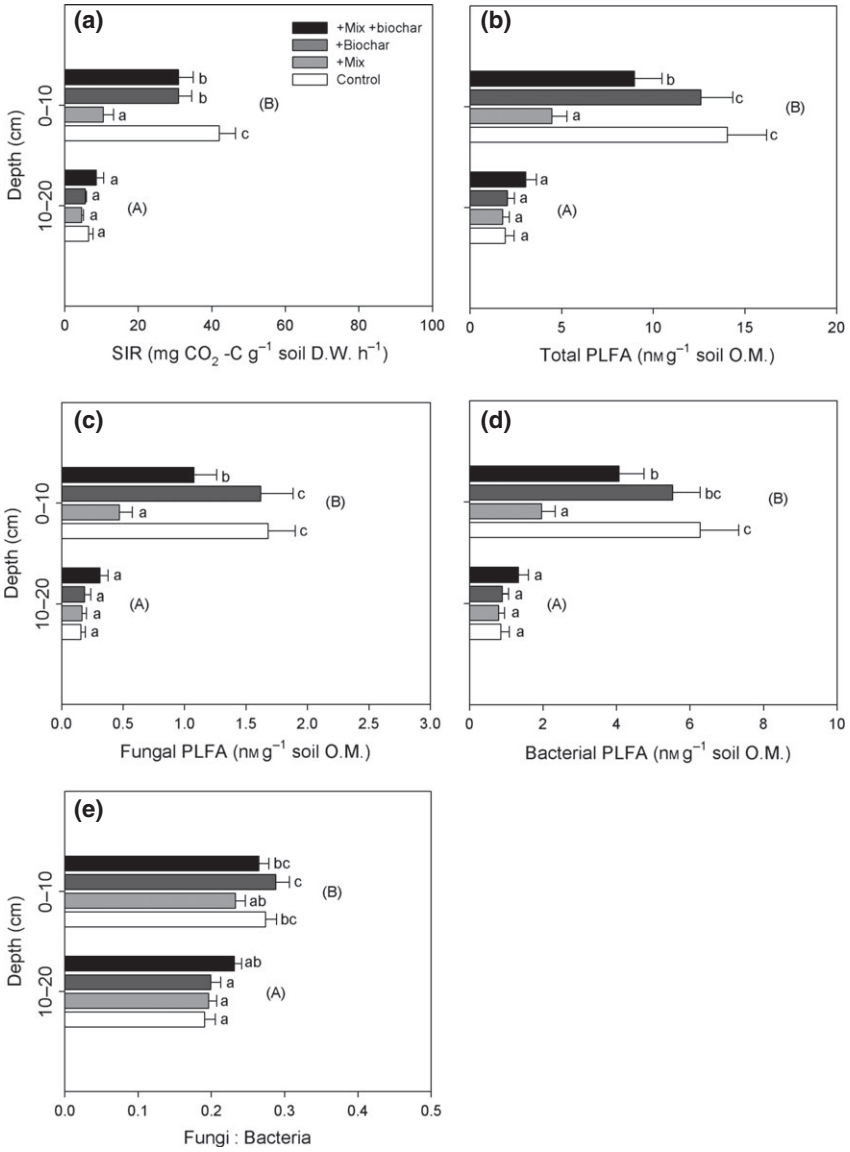

Fig. 3 The mean (±SE) substrate-induced respiration (SIR) rate (a), concentrations of total (b), fungal (c), and bacterial (d) phospholipid fatty acids (PLFAs), and the fungal:bacterial ratio (e) in response to soil depth (0–10 or 10–20 cm) and a factorial combination of biochar application and soil mixing. Within each panel, different capital letters (A or B) above each group of bars indicates a significant difference between the soil depths, whereas lower case letters (a or b) indicate significant differences across all bars, as indicated by *post hoc* Student–Neuman–Keuls tests.

rates independently of the soil mixing treatment), we found that biochar increased NH_4^+ availability, regardless of whether it was applied to the surface or mixed into the soil. However, a majority of other variables were unresponsive to biochar addition, such as NO_3^- and PO_4^--P, most microbial community variables, and soil respiration. One potential explanation for the observed increase in NH_4^+ concentrations is that the biochar itself served as a source of NH_4^+ , which accumulates within ash residues on its surfaces during the charring process (Gundale & Deluca, 2006; Pluchon *et al.*, 2014). However, if we consider that the mean soil bulk density at the site was 1 g cm^{-3} and a depth of 20 cm, the increase in NH_4^+ concentrations caused by biochar addition scales up to approximately 0.5 kg NH_4^+ per plot, which is 724 times greater than the NH_4^+ directly contributed by the biochar itself (i.e., 500 kg of biochar per plot × 1.38 mg NH_4^+ kg^{-1} biochar = 690 mg NH_4^+ added per plot). Thus, the positive effect of biochar on soil NH_4^+ concentrations was instead likely to have arisen by promoting net N mineralization rather than ash input (indicated by a main biochar

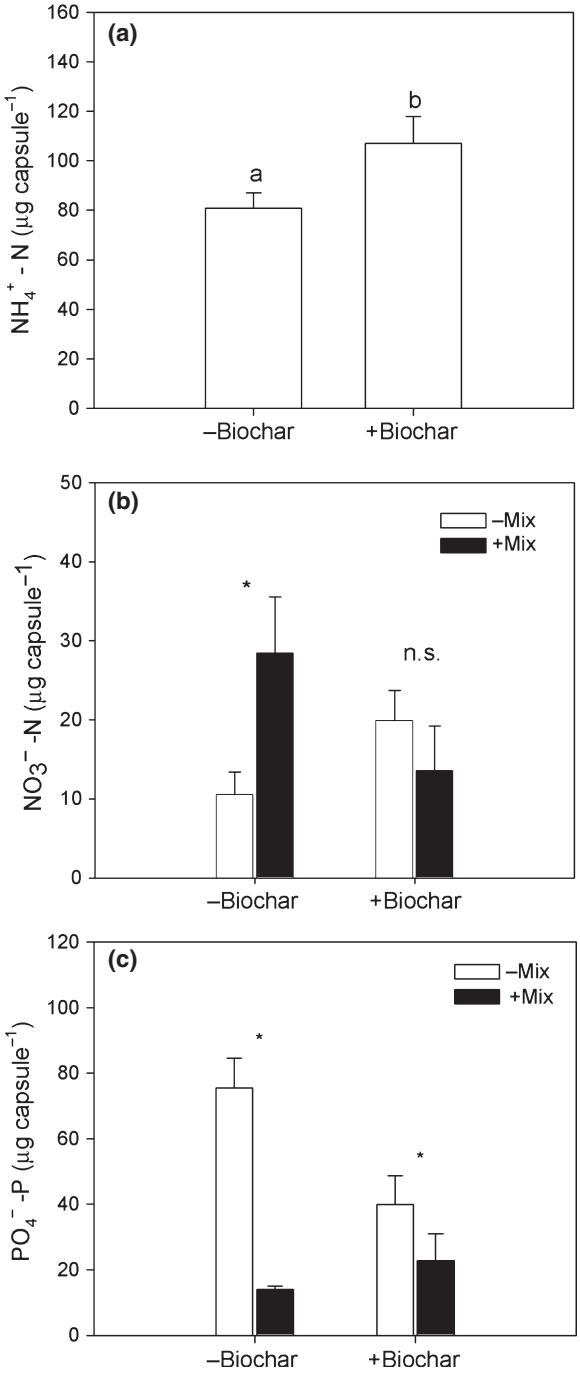

Fig. 4 The mean (±SE) resin-sorbed NH_4^+ in response to biochar addition (a), and resin-sorbed NO_3^- (b) and PO_4^- (c) in response to biochar application and soil mixing treatments. Within each subpanel different lower case letters above bars, or stars (*) above bar pairs indicate significant differences, as determined using *post hoc* Student's t-tests.

effect, Table 1), which was particularly evident in the mixed plots (indicated by a biochar by mixing interactive effect, Table 1). Enhancement of N mineralization associated with biochar especially in the mixed plots may have occurred as a result of enhanced gas exchange or soil moisture retention associated with its very high micropore volume (Keech *et al.*, 2005; Pluchon *et al.*, 2014).

Contrary to the first part of our second hypothesis (i.e., that soil mixing alone would enhance nutrient availability and mineralization rates, and soil respiration, but decrease total microbial biomass and fungal to bacteria ratios), we found that soil mixing caused a decline in a majority of these variables. While all plots within our experiment were subjected to a relatively minor level of disturbance during forest harvest and stump removal (which left the organic horizon mostly intact), our mixing treatment caused a substantially more severe disturbance by turning over the entire soil volume to a depth of approximately 30 cm, thereby likely affecting a wide range of soil properties such as aggregate structure, porosity, and gas exchange. The disturbance caused by the mixing treatment resembled several mechanical site preparation techniques (e.g., mounding or furrowing) widely employed in the boreal region used to enhance forest establishment, which have been shown in numerous studies to enhance short term nutrient availability and mineralization rates, as well as bacterial biomass, and soil respiration rates (Frey *et al.*, 2003; Siira-Pietikainen *et al.*, 2003; MacKenzie *et al.*, 2005; Piirainen *et al.*, 2007). These changes can occur because soil disturbance causes soil organic matter stabilized within soil aggregates to become more available to the microbial community, leading to short-term increase in bacterial biomass, soil respiration, nutrient transformation rates, and nutrient accumulation in the soil (Siira-Pietikainen *et al.*, 2003), followed by an eventual decline (Piirainen *et al.*, 2007). One potential explanation for the contradictory results found in our study is that these effects are short lived and had ended prior to our sampling effort.

We found no evidence for our second hypothesis that soil mixing would enhance effects of biochar on measured soil variables. Instead, we found that biochar dampened many of the impacts that mixing by itself had on soil nutrient availability, nutrient transformation rates, and microbial community variables. Several properties of biochar may have contributed to this dampening. For instance, numerous studies have shown that biochar can enhance microbial biomass and activity by serving as a substrate and habitat for microbes, and by improving soil physical properties (Pietikainen *et al.*, 2000; Lehmann *et al.*, 2011). Our finding of enhancement of SIR and soil bacterial and fungal PLFAs in the mixed soil with vs. without biochar would have likely led to the enhancement of net N mineralization rates, given

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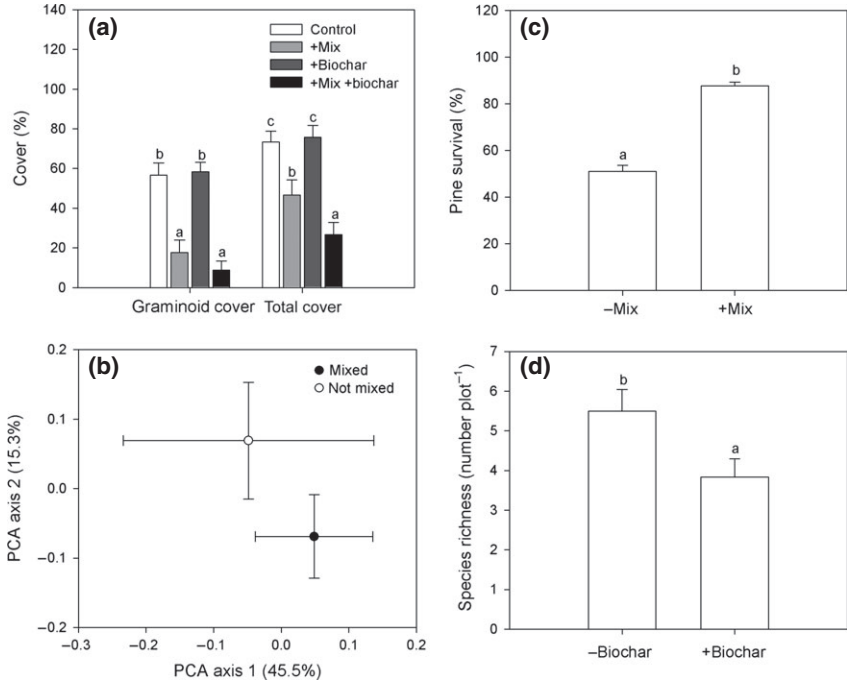

Fig. 5 The mean (±SE) graminoid and total cover of the ground layer vegetation in response to soil mixing and biochar treatments (a), the mean (±95% confidence interval) of the first and second axis of a principle component analysis (PCA) describing the vegetation composition (b), and mean (±SE) survival of *P. sylvestris* seedlings (c) in response to soil mixing treatments, and the response on species richness to the charcoal treatment (d). Different letters above bars (a or b) in subpanels a, c, and d indicate significant differences, as determined using Student–Neuman–Keuls *post hoc* tests (a) or analysis of variance (c and d).

Table 4 The *F*- and *P*-values from ANOVA evaluating the main and interactive effects of biochar (added or not added) and soil mixing (mixed or not mixed) on plant species richness, PCA axis 1 and 2, graminoid and total cover, and pine survival. Bolded *F*- and *P*- values indicate a significant effect at $P = 0.05$

	Mixing (M)*		Biochar (B)*		B × M*	
	F	*P*	*F*	*P*	*F*	*P*
Species Richness	2.1	0.166	**8.5**	**0.011**	0.1	0.775
Total cover (%)	**62.4**	**0.000**	3.3	0.088	**5.5**	**0.033**
Graminoid cover (%)	**117.3**	**0.000**	0.8	0.394	1.7	0.218
PCA 1	1.0	0.345	0.5	0.495	0.0	0.960
PCA 2	**8.4**	**0.011**	0.0	0.971	1.1	0.314
Pine Survival (%)	**145.9**	**0.000**	0.2	0.630	2.5	0.137

*Degrees of freedom: 1,5.

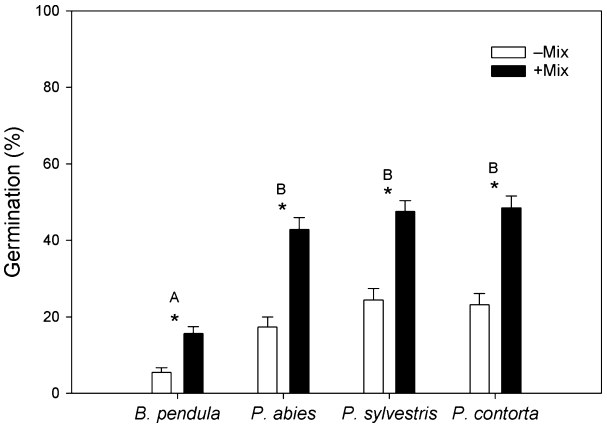

Fig. 6 The mean (±SE) germination rate (%) for planted seeds of *Betula pendula*, *Picea abies*, *Pinus sylvestris*, and *Pinus contorta* in response to soil mixing 92 days after seed sowing. Stars above each pair of bars indicate a significant difference, as determined using *post hoc* Student's t-tests whereas, capital letters above each bar pair indicate a significant difference in germination rate across the four species, as determined using Student–Neuman–Keuls *post hoc* tests.

this process is driven by the soil microbial community. This stimulation of the soil microbial community may also have immobilized NO_3^-, thus leading to the lower NO_3^- concentrations we observed in the mixed soil with vs. without biochar. The significant increase in soil PO_4^--P in mixed soils with vs. without biochar could have been due to biochar interfering with PO_4^- -P complexation into humic substances, or with Fe and Al ions (Lehmann *et al.*, 2003; Topoliantz *et al.*, 2005), which can limit P availability in the boreal region (Giesler *et al.*, 2005).

Table 5 The degrees of freedom, *F*- and *P*-values from a split plot ANOVA evaluating the main and interactive effects of biochar (added or not added), soil mixing (mixed or not mixed), seedling species, and their interactions on the percent germination of four seedling species (*Betula pendula*, *Picea abies*, *Pinus sylvestric*, and *Pinus contorta*). Bolded *F*- and *P*-values indicate a significant effect at $P = 0.05$

	df	*F*-value	*P*-value
Mixing	1,20	**23.4**	**<0.001**
Biochar	1,20	2.4	0.131
Species	3,441	**187.6**	**<0.001**
Mixing*Biochar	1,20	0.109	0.745
Biochar*Species	3,441	0.070	0.976
Mixing*Species	3,441	**5.215**	**0.002**
Mixing*Biochar*Species	3,441	0.403	0.751

Contrary to our third hypothesis (i.e., that treatment effects on nutrient availability would correspond with differences in plant community composition), we found that elevated soil NH_4^+ concentrations that occurred in response to biochar addition did not correspond with large changes to the ground layer vegetation. The only effect of biochar was to cause a small decrease in plant species richness per unit area. However, in partial support of our third hypothesis, we found that mixing reduced graminoid and total plant cover and promoted dominance by ericaceous shrubs; this corresponded with the lower net N mineralization rates and resin-sorbed PO_4^- -P concentrations in the mixed plots. The most likely explanation for the observed unresponsiveness of vegetation to biochar application, as well as for the relatively weak relationship between vegetation cover and nutrient availability, is that vegetation appeared to be primarily controlled by disturbance rather than soil nutrient availability over the time scale of our study. The soil mixing treatments clearly damaged and buried vegetation, resulting in a direct reduction in vegetation cover. While no effect of enhanced nutrient availability on vegetation was evident in our study, we anticipate that these effects could emerge over longer times scales, as the relative importance of plant competition for nutrients gradually becomes more important (Grime, 1979).

Contrary to the predictions of our fourth hypothesis, our data showed that biochar application had no discernable short-term impact on tree seed and seedling response despite significantly increasing extractable NH_4^+ concentrations. This result is consistent with some previous studies in northern conifer forests (Naydenov *et al*., 2006; Heiskanen *et al*., 2013) but not others (Wardle *et al*., 1998; Nilsson *et al*., 2008; Pluchon *et al*., 2014). Pluchon *et al.* (2014) showed that biochar effects on seedling growth of boreal forest tree species were determined by interactive effects between biochar and soil, whereby biochar with higher concentrations of available PO_4^- -P had large effects only in soils where P was limiting relative to N. This result could explain the unresponsiveness of seedlings to biochar application, as biochar may not have alleviated the primary resource limitation or reduced competition with the ground layer vegetation at the site over the time scale of our study. However, in support of our fourth hypothesis, *P. sylvestris* seedling growth and germination rates of four sown species (*P. sylvestris*, *P. contorta*, *P. abies*, and *B. pendula*) were strongly impaired in the plots where vegetation cover was the greatest (i.e., the unmixed plots). These results highlight the importance of ground layer vegetation in determining tree establishment success in boreal forests (Thiffault *et al*., 2013).

The suggestion that biochar management can be used to effectively mitigate global anthropogenic CO_2 emissions require its successful implementation in nearly every major biome, and thus, far studies evaluating biochar application in forest environments are almost completely lacking. Our study provides several insights into understanding the impacts and effectiveness of biochar management in forested environments and boreal forests specifically. First, it suggests that biochar application can enhance availability of some soil nutrients known to limit productivity in the boreal region (notably NH_4^+) while directly contributing to a relatively stable pool of soil C. Given that we applied 10 t ha^{-1} of biochar with a C content of 74% (i.e., 7.4 Mg C ha^{-1}) to a soil containing approximately five times as much C (i.e., 38.8 Mg ha^{-1}), and no changes in soil C efflux (i.e., soil respiration) were detected, our study suggests that biochar was relatively stable and at most caused only minor losses of existing soil C. This finding suggests that biochar may indeed serve as a useful tool to simultaneously enhance soil fertility and soil C stocks in boreal forests. Secondly, our data provide new insights into the potential impacts of biochar on aboveground C sequestration in the boreal region. Biochar alone was ineffective at enhancing growth of the ground layer vegetation or the regeneration of forest trees over the 2-year duration of our study, contrary to a wide range of studies conducted in agro-ecosystems that have shown positive effects on plant growth (Jeffery *et al*., 2011). Given that trees eventually account for most of the aboveground C in establishing forest stands, our results suggest that biochar application in the boreal region might be more useful and successful as a C storage and sequestration tool if used in combination with site preparation systems aimed at optimizing forest regeneration. Within this context, biochar may help prolong the short-term transient effects that these preparation techniques may have on soil nutrient availability, microbial

biomass, and activity, while directly enhancing recalcitrant soil C stocks. While our study covered a relatively short response period relative to a typical forest rotation length, it is a critical first step in evaluating the impacts of biochar management in forested ecosystems on the global C cycle. Our study, in combination with additional long-term studies, is necessary before biochar management can be accepted and promoted within proposed C trading schemes in the boreal region.

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References

Ameloot N, Sleutel S, Das KC, Kanagaratnam J, De Neve S (2015) Biochar amendment to soils with contrasting organic matter level: effects on N mineralization and biological soil properties. *GCB Bioenergy*, **7**, 135–144.

Anderson JPE, Domsch KH (1978) Physiological method for quantitative measurement of microbial biomass in soils. *Soil Biology & Biochemistry*, **10**, 215–221.

Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and Soil*, **337**, 1–18.

Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *Global Change Biology Bioenergy*, **5**, 202–214.

Bligh EG, Dyer WJ (1959) A rapid method to total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911–917.

Brewer CE, Unger R, Schmidt-Rohr K, Brown RC (2011) Criteria to select biochars for field studies based on biochar chemical properties. *Bioenergy Research*, **4**, 312–323.

Burney JA, Davis SJ, Lobell DB (2010) Greenhouse gas mitigation by agricultural intensification. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 12052–12057.

Chan KY, Zhihong X (2009) Biochar: nutrient properties and their enhancement. In: *Biochar for Environmental Management* (eds Lehmann J, Joseph S), pp. 67–81. Earthscan, Sterling, Virginia.

Clough TJ, Condron LM, Kammann C, Müller C (2013) A review of biochar and soil nitrogen dynamics. *Agronomy*, **3**, 275–293.

Cornwell WK, Cornelissen JHC, Amatangelo K, *et al.* (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecology Letters*, **11**, 1065–1071.

Cusack DF, Axsen J, Shwom R, Hartzell-Nichols L, White S, Mackey KRM (2014) An interdisciplinary assessment of climate engineering strategies. *Frontiers in Ecology and the Environment*, **12**, 280–287.

DeLuca TH, MacKenzie MD, Gundale MJ, Holben WE (2006) Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. *Soil Science Society of America Journal*, **70**, 448–453.

Eno CF (1960) Nitrate production in the field by incubating the soil in polyethylene bags. *Soil Science Society of America Proceedings*, **24**, 277–299.

Frey BR, Lieffers VJ, Munson AD, Blenis PV (2003) The influence of partial harvesting and forest floor disturbance on nutrient availability and understory vegetation in boreal mixedwoods. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **33**, 1180–1188.

Giesler R, Andersson T, Lovgren L, Persson P (2005) Phosphate sorption in aluminum- and iron-rich humus soils. *Soil Science Society of America Journal*, **69**, 77–86.

Glaser B, Haumaier L, Guggenberger G, Zech W (2001) The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. *Naturwissenschaften*, **88**, 37–41.

Grime JP (1979) *Plant Strategies and Vegetation Processes*. John Wiley and Sons, London, UK.

Gundale MJ, Deluca TH (2006) Temperature and source material influence ecological attributes of ponderosa pine and Douglas-fir charcoal. *Forest Ecology and Management*, **231**, 86–93.

Gundale MJ, Deluca TH, Nordin A (2011a) Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. *Global Change Biology*, **17**, 2743–2753.

Gundale MJ, Fajardo A, Lucas RW, Nilsson M-C, Wardle DA (2011b) Resource heterogeneity does not explain the productivity-diversity relationship across a boreal island fertility gradient. *Ecography*, **34**, 887–896.

Gundale MJ, From F, Bach LH, Nordin A (2014) Anthropogenic nitrogen deposition in boreal forests has a minor impact on the global carbon cycle. *Global Change Biology*, **20**, 276–286.

Gundale MJ, Sutherland S, Deluca TH (2008) Fire, native species, and soil resource interactions influence the spatio-temporal invasion pattern of *Bromus tectorum*. *Ecography*, **31**, 201–210.

Hasselquist NJ, Metcalfe DB, Hogberg P (2012) Contrasting effects of low and high nitrogen additions on soil CO2 flux components and ectomycorrhizal fungal sporocarp production in a boreal forest. *Global Change Biology*, **18**, 3596–3605.

Heiskanen J, Tammeorg P, Dumroese RK (2013) Growth of Norway spruce seedlings after transplanting into silty soil amended with biochar: a bioassay in a growth chamber - Short Communication. *Journal of Forest Science (Prague)*, **59**, 125–129.

Hobbie SE (1996) Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecological Monographs*, **66**, 503–522.

IPCC (2013) Intergovermental Panel on Climate Change Fourth Assessment Report: Working Group 1 contribution to the firth assessment report of the intergovernmental panel on climate change, Chapter 11. In: *Climate Change 2013: The physical science basis*. (eds Stocker TF, Qin D), pp. 1–119. New York, NY, Cambridge University Press.

Jeffery S, Bezemer TM, Cornelissen G, *et al.* (2013) The way forward in biochar research: targeting trade-offs between the potential wins. *Global Change Biology Bioenergy*, **7**, 1–13.

Jeffery S, Verheijen FGA, Van Der Velde M, Bastos AC (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture Ecosystems & Environment*, **144**, 175–187.

Jones DL, Murphy DV, Khalid M, Ahmad W, Edwards-Jones G, Deluca TH (2010) Short-term biochar-induced increase in soil CO(2) release is both biotically and abiotically mediated. *Soil Biology & Biochemistry*, **43**, 1723–1731.

Keech O, Carcaillet C, Nilsson M-C (2005) Adsorption of allelopathic compounds by wood-derived charcoal: the role of wood porosity. *Plant and Soil*, **272**, 291–300.

Lehmann J (2007a) Bio-energy in the black. *Frontiers in Ecology and the Environment*, **5**, 381–387.

Lehmann J (2007b) A handful of carbon. *Nature*, **447**, 143–144.

Lehmann J, Da Silva JP Jr, Steiner C, Nehls T, Zech W, Glaser B (2003) Nutrient availability and leaching in an archaeological Anthrosol and a Ferrasol of the Central Amazon basin: fertilizer, manure, and charcoal amendments. *Plant and Soil*, **249**, 343–357.

Lehmann J, Joseph S (eds) (2009) *Biochar for Environmental Management*. Earthscan, Sterling, Virgina.

Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects of soil biota - A review. *Soil Biology & Biochemistry*, **43**, 1812–1836.

Liang B, Lehmann J, Sohi SP, *et al.* (2010) Black carbon affects the cycling of non-black carbon in soil. *Organic Geochemistry*, **41**, 206–213.

Lindholm EL, Stendahl J, Berg S, Hansson PA (2011) Greenhouse gas balance of harvesting stumps and logging residues for energy in Sweden. *Scandinavian Journal of Forest Research*, **26**, 586–594.

Liu XY, Zhang AF, Ji CY, *et al.* (2013) Biochar's effect on crop productivity and the dependence on experimental conditions-a meta-analysis of literature data. *Plant and Soil*, **373**, 583–594.

MacKenzie MD, Schmidt MG, Bedford L (2005) Soil microclimate and nitrogen availability 10 years after mechanical site preparation in northern British Columbia. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **35**, 1854–1866.

McIntosh ACS, Macdonald SE, Gundale MJ (2012) Tree species versus regional controls on ecosystem properties and processes: an example using introduced Pinus contorta in Swedish boreal forests. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **42**, 1228–1238.

Naydenov K, Tremblay F, Bergeron Y, Goudiaby V (2006) Influence of active charcoal on the primary growth of jack pine seedlings. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **36**, 761–767.

Nelissen V, Rütting T, Huygens D, Staelens J, Ruysschaert G, Boeckx P (2012) Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. *Soil Biology and Biochemistry*, **55**, 20–27.

 doi: 10.1111/gcbb.12274

Nilsson MC, Wardle DA, Deluca TH (2008) Belowground and aboveground consequences of interactions between live plant species mixtures and dead organic substrate mixtures. *Oikos*, **117**, 439–449.

O'Neill B, Grossman J, Tsai MT, *et al.* (2009) Bacterial community composition in Brazilian Anthrosols and adjacent soils characterized using culturing and molecular identification. *Microbial Ecology*, **58**, 23–35.

Ohlson M, Dahlberg B, Okland T, Brown KJ, Halvorsen R (2009) The charcoal carbon pool in boreal forest soils. *Nature Geoscience*, **2**, 692–695.

Palm CA, Smukler SM, Sullivan CC, Mutuo PK, Nyadzi GI, Walsh MG (2010) Identifying potential synergies and trade-offs for meeting food security and climate change objectives in sub-Saharan Africa. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 19661–19666.

Pietikainen J, Kiikkila O, Fritze H (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos*, **89**, 231–242.

Piirainen S, Finer L, Mannerkoski H, Starr M (2007) Carbon, nitrogen and phosphorus leaching after site preparation at a boreal forest clear-cut area. *Forest Ecology and Management*, **243**, 10–18.

Pluchon N, Gundale MJ, Nilsson MC, Kardol P, Wardle DA (2014) Stimulation of boreal tree seedling growth by wood-derived charcoal: effects of charcoal properties, seedling species and soil fertility. *Functional Ecology*, **28**, 766–775.

Post WM, Izaurralde RC, West TO, Liebig MA, King AW (2012) Management opportunities for enhancing terrestrial carbon dioxide sinks. *Frontiers in Ecology and the Environment*, **10**, 554–561.

Preston CM, Schmidt MWI (2006) Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. *Biogeosciences*, **3**, 397–420.

Robertson SJ, Rutherford PM, Lopez-Gutierrez JC, Massicotte HB (2012) Biochar enhances seedling growth and alters root symbioses and properties of sub-boreal forest soils. *Canadian Journal of Soil Science*, **92**, 329–340.

Sabatti M, Fabbrini F, Harfouche A, *et al.* (2014) Evaluation of biomass production potential and heating value of hybrid poplar genotypes in a short-rotation culture in Italy. *Industrial Crops and Products*, **61**, 62–73.

Sabine CL, Heinmann M, Artaxo P *et al.* (2004) Current status and past trends on the global carbon cycle. In: *The Global Cycle SCOPE 62*. (eds Field CB, Raupach MR), pp. 17–44. Island Press, Washington, DC.

Siira-Pietikainen A, Haimi J, Fritze H (2003) Organisms, decomposition, and growth of pine seedlings in boreal forest soil affected by sod cutting and trenching. *Biology and Fertility of Soils*, **37**, 163–174.

Sohi SP (2012) Carbon storage with benefits. *Science*, **338**, 1034–1035.

Steiner C, Teixeira WG, Lehmann J, Nehls T, De Macedo JLV, Blum WEH, Zech W (2007) Long term effects of manure, charcoal, and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil*, **291**, 275–290.

Thiffault N, Fenton NJ, Munson AD, *et al.* (2013) Managing understory vegetation for maintaining productivity in black spruce forests: a synthesis within a multiscale research model. *Forests*, **4**, 613–631.

Topoliantz S, Pong J-F, Ballof S (2005) Manioc peel and charcoal: a potential organic amendment for sustainable soil fertility in the tropics. *Biology and Fertility of Soils*, **41**, 15–21.

Walmsley JD, Godbold DL (2010) Stump harvesting for bioenergy - a review of the environmental impacts. *Forestry*, **83**, 17–38.

Wardle DA (1993) Changes in the microbial biomass and metabolic quotient during leaf-litter succession in some New Zealand forest and scrubland ecosystems. *Functional Ecology*, **7**, 346–355.

Wardle DA, Gundale MJ, Jäderlund A, Nilsson M-C (2013) Decoupled long-term effects of nutrient enrichment on aboveground and belowground properties in subalpine tundra. *Ecology*, **94**, 904–919.

Wardle DA, Hornberg G, Zackrisson O, Kalela-Brundin M, Coomes DA (2003) Long-term effects of wildfire on ecosystem properties across an island area gradient. *Science*, **300**, 972–975.

Wardle DA, Jonsson M, Bansal S, Bardgett RD, Gundale MJ, Metcalfe DB (2012) Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long term natural experiment. *Journal of Ecology*, **100**, 16–30.

Wardle DA, Nilsson MC, Zackrisson O (2008) Fire-derived charcoal causes loss of forest humus. *Science*, **320**, 629–630.

Wardle DA, Zackrisson O, Nilsson MC (1998) The charcoal effect in boreal forests: mechanisms and ecological consequences. *Oecologia*, **115**, 419–426.

Zackrisson O, Nilsson M-C, Wardle DA (1996) Key ecological function of charcoal from wildfire in the boreal forest. *Oikos*, **77**, 10–19.

Zar JH (1999) *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, NJ.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The mean (±SE) soil respiration rates in response to soil mixing, measured at six sampling times during the growing season, 2013.